

**Amendments to the Specification**

Please replace the paragraph on page 11, lines 19-20, with the following paragraph:

Figure 3 depicts the vector (and its multiple cloning site) used to prepare the exemplary oriented fragment cDNA library described herein (SEQ ID NO: 13).

Please replace the paragraphs on page 14, line 31 to page 15, line 23 with the following paragraphs:

**4. Oligonucleotides and adaptors.**

**a. Adaptors and oligonucleotides.**

The following oligonucleotides constitute the adaptor (CSAD - cap side adaptor) that will ligate to the 3' end (the CAP side with regard to the orientation of the mRNA) of the sscDNA.

CSN4C2-A 5'-GCCATTAAGGCCACCATGCCNNNN-3'Block 24mer (SEQ-ID NO: 1)

CSAD-A 5'-p-CATGGTGGCCTTAATGGCCACTACGACCGTTCGGGTGGTAC-3'Block 41mer (SEQ ID NO: 2)

The structure of the CSAD adaptor after annealing is:

CSN4C2-A 5'-GCCATTAAGGCCACCATGCCNNNN-3'Block (SEQ ID NO: 3)

CSAD-A 3'-CATGGTGGGCTTGCCAGCATCACCGGTAATTCCGGTGGTAC-P-5' (SEQ ID NO: 4)

The following oligonucleotides constitute the adaptor (PSAD - poly A side adaptor) that will ligate to the 5' end (the polyA side with regard to the orientation of the mRNA) of the sscDNA.

PSN4G2 5'-NNNNGGTGAGTGACTGAGGCC-3'Block 21mer (SEQ ID NO: 5)

Applicants: Paz Einat, et al.  
U.S. Serial No.: 10/561,005  
Filed: as §371 national stage of PCT  
International Application No. PCT/IL2004/000515  
Page 3

PSAD 5'-CGAGGAGCGACCGACTCGATGGCCGAGGCGCCTCAGTCACTCA-3'  
44mer (SEQ ID NO: 6)

The structure of the PSAD adaptor after annealing is:

PSN4G2 5'-NNNNGGTGAGTGACTGAGGCC-3' Block (SEQ ID NO: 7)

PSAD 3'-ACTCACTGACTCCGGCGGAGCCGGTAGCTCAGCCAGCGAGGAGC-5'  
(SEQ ID NO: 8)

b. Oligonucleotide annealing to form the adaptors.

100µm from each oligonucleotide of the pair that constitute an adaptor was mixed in 25µl with an annealing buffer of 10mM Tris-HCl, 7mM MgCl<sub>2</sub>, 100mM NaCl.

The mix was placed in a 70°C water bath previously switched off to permit cooling to room temperature.

Please replace the paragraphs on page 15, line 33 to page 16, line 16 with the following paragraphs:

#### **6. PCR amplification of the adaptor ligated sscDNA.**

The oligonucleotides used for PCR were:

CSPCR 5'-GTACCACCCGAACGGTCGTAG-3' 21mer (for CSAD) (SEQ ID NO: 9)

PSPCR 5'-CGAGGAGCGACCGACTCGATG-3' 21mer (for PSAD) (SEQ ID NO: 10)

The PCR was performed in two steps so as to avoid formation of an adaptor dimer that could interfere with the amplification.

a. "Pre-PCR" for 3 cycles, without purification of the ligation reaction. A reaction volume of 100µl included 15µl of the ligation reaction, the PCR primers that match the adaptors: CSPCR and PSPCR oligonucleotides (final concentration of 0.4mM),

0.2mM dNTPs, ExTaq buffer and 3 units of ExTaq polymerase (TaKaRa).

PCR cycling parameters were: 95°C 2', 95°C 30'', 63°C 30'', 72°C 1 min, 3 cycles, 63°C 10', hold 4°C.

b. The "pre-PCR" products were purified on GeneClean III and eluted in 30µl 1 mM Tris pH 8.5.

c. Preparative PCR. Reaction volume of 50µl included 27µl of the purified "pre-PCR" products and the same reaction constituents as in "a". PCR cycling parameters were: 95°C 2', 95°C 30'', 63°C 30'', 72°C 1 min, 8 cycles, 63°C 10', hold 4°C. PCR products were precipitated with EtOH in the presence of 20µg glycogen carrier. The pellet was dissolved in 20 µl DDW.

Please replace the paragraphs on page 16, line 32 to page 17, line 17 with the following paragraphs:

#### **9. Description of cloning strategy using Sfi I.**

Ligated cDNA from each fraction was digested with Sfi I in preparation for ligation into the vector. Sfi I cleaves the following recognition sequence:

5' ... G G C C N N N N<sup>^</sup>N G G C C ... 3' (SEQ ID NO: 11)  
3' ... C C G G N<sup>^</sup>N N N N C C G G ... 5' (SEQ ID NO: 12)

Thus, it generates a 3-basepair overhang of a sequence that is not part of the recognition sequence. This feature was utilized to generate two different cloning specificities in the two ends of the ligated cDNA. The CSAD generates an over-hang of:

5'AGGCC---  
3'AATTCGG---

while the PSAD adaptor generates an over-hang of:

---GGCCCGGA-3'  
---CCGGG-5'

Applicants: Paz Einat, et al.  
U.S. Serial No.: 10/561,005  
Filed: as §371 national stage of PCT  
International Application No. PCT/IL2004/000515  
Page 5

Two matching Sfi I recognition sites were introduced into the cloning expression-vector pLNCX2 so that the PSAD side will be close to the promoter region in the cloning vector and the CSAD side will be close to the polyA region. This will cause the cDNA inserts to be in the ANTISENSE orientation in the cloning expression-vector.

Another advantage of using Sfi I is that its recognition sequence is composed of HaeIII cleavage recognition site (GGCC) and thus Sfi I will not cleave within the ligated cDNA (no internal SfiI sites are left and thus, no fragment is lost).